

article, microorganism, blood cell and cell membrane fragment, said insoluble carrier being capable of aggregation;

(b) an enzyme inhibitor for reacting with and inhibiting activity of said enzyme, said enzyme inhibitor being in a free state uncoupled to an antigen or antibody and being increasingly unhindered from reacting with said enzyme when said insoluble carrier is increasingly agglutinated; and

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(c) a substrate for the enzyme capable of producing an optically detectable indication of reaction with the enzyme, wherein said substrate is not hindered from reacting with said enzyme when said enzyme is unreacted with said enzyme inhibitor when said insoluble carrier is increasingly aggregated, said components (a) - (c) being maintained separate and apart and mixed together only with a sample containing the target antigen or antibody.

2. (Twice Amended) An immunoassay reagent for use in a quantitative determination of a target antigen or antibody present in a sample, said reagent consisting essentially of the following components:

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(a) an insoluble carrier which carries and is coupled to an enzyme and an antibody or antigen reactive with said target antigen or antibody, said insoluble carrier comprising at least one selected from the group consisting of an organic polymer powder article, microorganism, blood cell and cell membrane fragment, said insoluble carrier being capable of aggregation;

(b) an enzyme inhibitor for reacting with and inhibiting activity of said enzyme, said enzyme inhibitor being in a free state uncoupled to an antigen or antibody and being increasingly hindered from reacting with said enzyme once that insoluble carrier is increasingly agglutinated; and

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(c) a substrate for the enzyme capable of producing an optically detectable indication of reaction with the enzyme, wherein said substrate is not hindered from reacting with said enzyme when said enzyme is unreacted with said enzyme inhibitor when said insoluble carrier is increasingly aggregated, said immunoassay reagent consisting of a first reagent and a second reagent, wherein said first reagent contains said insoluble carrier in (a) above, and said second reagent contains said enzyme inhibitor and said substrate in (b) and (c) above.

3. (Fourth Amended) An immunoassay reagent for use in quantitative determination of a target antigen or antibody present in a sample, said reagent consisting essentially of the following components:

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(a) an insoluble carrier which carries and is coupled to an enzyme inhibitor and an antibody or antigen reactive with said target antigen or antibody, said insoluble carrier comprising at least one selected from the group consisting of an organic polymer powder particle, microorganism, blood cell and cell membrane fragment, said insoluble carrier being capable of aggregation;

(b) an enzyme which reacts with and whose activity is

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inhibited by said enzyme inhibitor, said enzyme being in a free state uncoupled to an antigen or antibody and the reaction between the enzyme and enzyme inhibitor being dependent upon the amount of antigen or antibody present in a sample, and in the presence of an antigen or antibody the insoluble carriers are caused to aggregate, resulting in stearic hindrance of resulting aggregates and reduction of reactions between the enzyme and enzyme inhibitor on the insoluble carrier; and

(c) a substrate with which the enzyme reacts, said components (a)-(c) being maintained separate and apart and sequentially mixed together only with a sample of target antigen or antibody, the addition of the substrate facilitating reaction with the enzyme, thereby effecting an optically detectable change in absorbence.
